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FORELEGS OF BUTTERFLIES I.

INTRODUCTION: CHEMORECEPTION.

RICHARD M. FOX

Carnegie Museum, Pittsburgh, Pa. 15213

This study, planned as the first of a short series on butterfly forelegs, considers some general questions raised by peculiarities of these appendages, briefly reviews evidence for chemoreception by the legs of butterflies and presents a preliminary investigation of an additional chemoreceptive function. Subsequent studies will be concerned with morphologic homologies, taxonomic correlations and with function.

It is a pleasure to acknowledge the assistance of Albert Lloyd, John Bauer, Richard T. Satterwhite — who also prepared the drawings — and Jean Walker Fox, all of Carnegie Museum, in collecting live material for study, and of Clifford F. Thompson, graduate student at the University of Pittsburgh, in preparing serial sections for me. I am particularly grateful to Dr. Vincent G. Dethier for reading a preliminary version of this paper and for his very useful suggestions.

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SOME GENERAL CONSIDERATIONS

The degree of reduction in size of the prothoracic legs and differences in reduction between the sexes have traditionally been among the principal characters used to define the major butterfly taxa — the four superfamilies as presented by Fox *et al.* (1965), sometimes ranked as families: Hesperioidea, Papilionoidea, Nymphaloidea and Lycaenoidea. My reasons for using superfamilies have been discussed elsewhere (Fox *et al.*, 1965: 44-46).

The primitive condition probably was one in which all three pairs of legs were of about the same size and each leg had all five tarsal subsegments and a post-tarus bearing claws and certain median structures (see Fox and Fox, 1964: 71-72). Among butterflies, reduction of forelegs occurs in two different ways:

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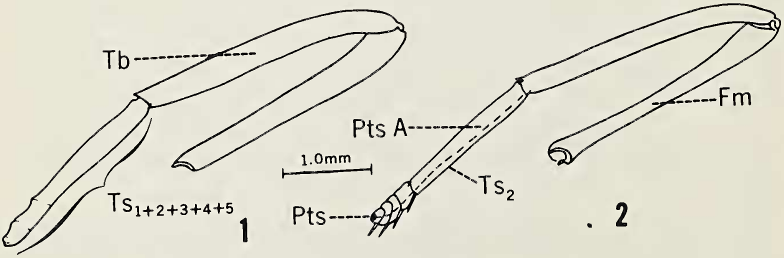
(1) miniaturization without loss of any part, and (2) miniaturization along with reduction through fusion in the apparent number of tarsal subsegments, plus the real or apparent loss of the post-tarus and, in a few extreme cases, reduction in proportionate size of the tibia.

Reduction is minimal among Hesperioidea and Papilionoidea. The prothoracic legs of both sexes are only slightly shorter than the mesothoracic and metathoracic legs and all "normal" parts of the tarsi and post-tarsi are present. To a slight degree, this is type 1 reduction.

In Lycaenoidea the females have the forelegs distinctly smaller than the mid- and hindlegs, but all five tarsal subsegments and the post-tarsus are present. The males not only have the forelegs quite small, but the post-tarsus is absent and some or all of the tarsal subsegments are fused. Thus females exhibit type 1 reduction while males have type 2 reduction.

Foreleg reduction is pronounced among Nymphaloidea, with marked miniaturization in both sexes. In males (Fig. 1) the post-tarsus is never present and, as a result of fusion, the tarsus superficially appears to have only one subsegment. In most nymphaloid families the female foreleg (Fig. 2), despite its minute size, retains all five tarsal subsegments and the post-tarsus, though the post-tarsal claws are absent in all but a few of the most primitive species. Again, type 2 reduction is found in males, type 1 in females.

Within each superfamily there is, of course, variation in leg ratios; this variation, which has useful taxonomic correlation, will be considered subsequently. Meanwhile, in order to illustrate



Figs. 1 and 2. Forelegs of *Vanessa atalanta*, coxae not shown, drawn to the same scale. Fig. 1, male; fig. 2, female.*Fm*, femur. *Pts*, post-tarsus. *PtsA*, post-tarsal apodeme (tendon). *Tb*, tibia. *Ts*, tarsus, with subscript numerals denoting subsegments.

in a general way the comparison among the four major taxa, the legs of both sexes of a typical species of each superfamily were measured and the pro- and methathoracic legs expressed as percentages of the length of the mesothoracic legs (Fig. 3).

The reduction of the forelegs of butterflies raises several interesting questions, one of which is that of selective value.

Many insects sacrifice ambulation in order to convert the forelegs to some other purpose having a clearly positive selective effect – mantids and mole crickets are among many possible examples. Such cases make it evident that the meso- and methathoracic legs suffice for ambulation; one may fairly assume that the forelegs can be altered and deprived of ambulatory function without *thereby* creating a negative selective effect. But may one properly conclude from this that the forelegs will actually be altered *unless* the alteration leads to a positive selective value? Certainly, there is no obvious selective advantage in the little forelegs of so many butterflies: is this a case of modification without either positive or negative selective value?

Apparently neutral, nonadaptive characters have been observed in animals and discussions to this point are found in most general books on evolution (e.g., Dodson, 1960: 250-251; Moody, 1953: 319, 328, 353-354). Dodson states that a character with truly neutral selective value can become established (1) through

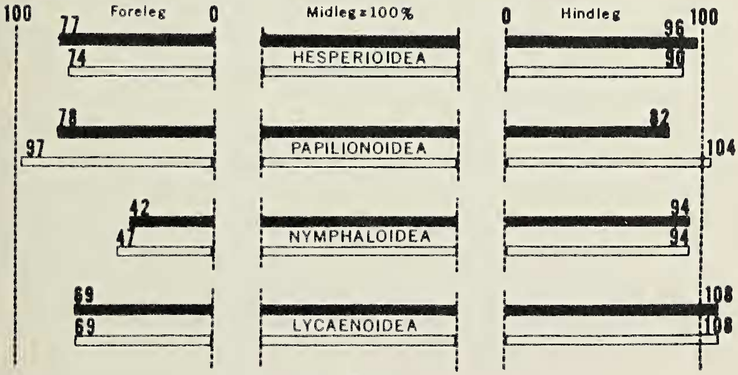


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Fig. 3. Ratio of length of fore- and hindlegs to midlegs of four African species selected at random; meaurements made from *Tagiades flesus* (Hesperiidae), *Papilio dardanus* (Papilionidae), *Cymothoe beckeri* (Nymphalidae) and *Iolaus agnes* (Lycaenidae). Males shown by solid bars, females by open bars. Lengths of midlegs taken as unity.

genetic drift, (2) as an incidental effect of a pleotropic gene, (3) because of linkage with a beneficial gene, or (4) as part of the phenomenon of vestigiality. I have previously commented (Fox, 1956: 24-25) that the strongly aborted forelegs of Ithomiidae and related families might become established *merely* because foreleg reduction is not itself detrimental to survival for these insects.

But Dodson (1960: 250) makes the point that nonadaptivity is difficult to demonstrate and that many characters, which at first seemed to be selectively neutral, have proved upon closer analysis to have positive adaptive value. Are the little forelegs of nymphaloids really useless? Have they indeed only neutral selective value or is there some positive value?

It is most intriguing that the degree and type of foreleg reduction differs between the sexes in two of the four superfamilies. Why do the tarsal subsegments and the post-tarsus persist in females in spite of radical miniaturization, whereas in males with equally miniaturized forelegs, the post-tarsus and most of the tarsal subsegments are absent? Are forelegs more useful to female butterflies than to males? If so, what function can be carried out by a miniaturized foreleg but requires all the segmental components and cannot be performed by a leg having the tarsal subsegments drastically fused and reduced?

SUCROSE CHEMORECEPTION

Other than ambulation, the only important function ever demonstarted for butterfly legs is the perception of fluids — specifically, water and water solutions of sugars — by means of contact chemoreceptors on the tarsi.

Experiments reported by Minnich (1921, 1922a, 1922b), Weis (1930), Anderson (1932), Frings and Frings (1949, 1956), Kuwabara (1951, 1952, 1953), and Hodgson (1958) used the "Proboscis reaction" to demonstrate that butterflies perceive fluids via tarsi and proboscis. Individual specimens were restrained and the proboscis and legs were put into contact with various fluids; if the proboscis was extended as for drinking, perception was considered confirmed.

Most of this work was physiologically oriented and primarily concerned with finding the lowest concentration of sugar in water which would elicit the reaction. Several workers also sought a "negative" reaction to solutions of materials thought to be unpleasant to the butterfly. In general, little effort was made to discriminate among the pairs of legs as to respective perception.

Thus, sensitivity on the midtarsi was reported for papilionids, lycaenids and hesperids studied but there was no indication as to whether or not the fore- or hindtarsi were separately checked.

However, Minnich (1921) and Frings and Frings (1949, 1956) obtained selective data on all three pairs of legs for two pierids and six nymphaloids. The pierids studied both showed positive reaction to solutions via the ventral surfaces of the fore- and midtarsi but no reaction via the hindtarsi. The nymphaloids showed positive reaction via the mid- and hindtarsi but negative via the foretarsi. These results suggest a taxonomic correlation of considerable interest.

Reported results of experiments with butterflies along these lines are summarized in Table 1.

Eltringham (1933), apparently at Minnich's behest, prepared celloidin sections of the midtarsus of male *Vanessa atalanta* and reported the presence of trichoid sensilla scattered along the ven-

Table 1

Perception of Solutions by Contact Chemoreception
Recorded for Butterfly Tarsi

Family, genus & species		Reaction to solutions by			Reference
		Foretarsus	Midtarsus	Hindtarsus	
Papilionidae:	*Papilio machaon*	?	+	?	4
	Papilio polyxenes	?	+	?	4
	Papilio philenor	?	+	?	5
	Papilio ajax	?	+	?	2
Pieridae:	*Colias philodice*	+	+	–	3
	Pieris rapae	+	+	–	2, 3
Danaidae:	*Danais plexippus*	–	+	+	2, 3
Satyridae:	*Lethe eurydice*	–	+	+	4
	Cercyonis pegala	–	+	+	2, 4
Nymphalidae:	*Liminitis arthemis*	–	+	+	4
	Liminitis archippus	?	+	?	2
	Vanessa atalanta	–	+	+	1, 2
	Nymphalis antiopa	?	+	+	1
	Phyciodes tharos	?	+	?	2
	Speyeria cybele	–	+	+	4
Lycaenidae:	*Lycaena thoe*	?	+	?	2
	Atlides halesus	?	+	?	5
Hesperiidae:	*Polites mystic*	?	+	?	2
	Epargyreus clarus	?	+	?	5

+, positive reaction reported. –, negative reaction reported. ?, no observation reported. References: 1, Minnich, 1921. 2, Anderson, 1932. 3, Frings & Frings, 1949. 4, Frings & Frings, 1956. 5, Hodgson, 1958.

tral surface. He described these structures as "extremely slender, thin-walled tubes . . . set in sockets of the usual kind" and supplied with a trichogen cell, a tormogen cell and with "several sensory cells . . . having a distal process . . . which enters the delicate tube on the edge of its base, and taking a somewhat irregular course, extends right to the distal end, where there in a minute papilla." The setae were found to be about 150 micra long and 6.2 micra in diameter. Grabowski and Dethier (1954) examined the mid- and hindlegs of a hesperid (*Epargyreus clarus*), a lycaenid (*Everes comyntas*), two pierids (*Colias eurytheme, Pieris rapae*) and a nymphalid (*Phyciodes tharos*) and found that "all possess on the tarsi only one type of thin-walled hair. This is blunt-tipped and usually curved, but the wall appears to be of equal thickness throughout." Foretarsi of females were not examined.

The supposition that these trichoid sensilla are the contact chemoreceptors concerned with detection of sugars and certain other solutions was confirmed by electrophysiological techniques by Morita and Takeda (1957, 1959), Morita, Doira, Takeda and Kuwabara (1957) and Takeda (1961).

Note that all reports on the morphology of tarsal chemoreceptors mentioned that these trichoid sensilla are scattered singly, and that no difference between the sexes — if, indeed, females were critically examined — was recorded.

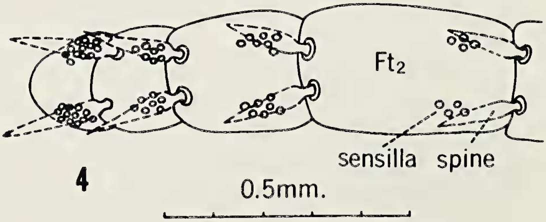

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Fig. 4. Second (*Ft* $_2$), third, fourth and fifth foretarsal segments of *Speyeria cybele* female in ventral view to show arrangement of trichoid sensilla and spines.

CLUMPED SENSILLA ON FEMALE FORETARSI

Many years ago I noticed that the foretarsi of female ithomids bear clusters of translucent hairs on the ventral surface. More recently, while trying to find some sound morphological basis for recognizing species in *Mechanitis,* a genus in which male genitalia are valueless for the purpose, I made a comparative examination of the female foretarsi and noticed that these clustered hairs are in fact the setae of trichoid sensilla and that the clusters are always placed beneath the spine or spines borne on the preceding subsegment.

Since all available information indicates that sucrose sensitivity is not vested in the foretarsi of nymphalids ,and since trichoid sensilla are known to be chemoreceptors, my curiosity was at once aroused. I then made whole mounts of the legs of several hundred butterflies representing all families and, of the Nymphaloidea, the principal subfamilies and tribes. Clustered trichoid sensilla were found on the foretarsi of all females of all groups, but such clusters were not found on foretarsi of males or on mid- or hindtarsi of any butterfly of either sex. (Details of the technique for whole mounts, which is quick, easy and permanent, are given in Cummins, Miller, Smith and Fox, 1965: 151-152.)

In order to verify the histological structure of these trichoid sensilla, legs were removed from freshly caught specimens of *Speyeria cybele,* fixed in Allen's modification of Bouin's fluid, embedded in high melting point (62°) wax, cut at 7 micra and stained for general examination with Mallory's triple stain.

On whole mounts of foretarsi of female nymphaloids, the trichoid sensilla (Figs. 4, 5, 6) are seen as groups of blunt-tipped,

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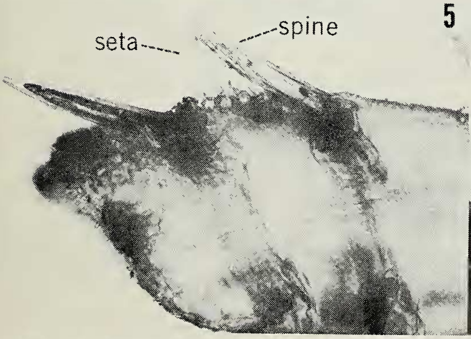

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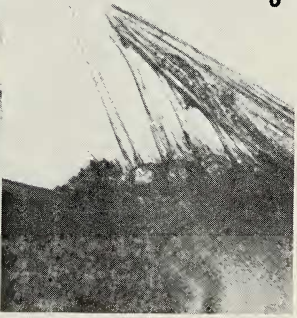

Fig. 5. Three terminal foretarsal subsegments of S. *cybele* female photographed in lateral view at 100x from a whole mount.

Fig. 6. Fourth foretarsal subsegment of same, photographed at 200x in lateral view.

translucent curved setae, each arising from the center of what appears to be a translucent disc or mound. These sensilla are arranged in tight clusters on the ventral side near the proximal end of a subsegment and beneath the ends of the large, prominent spines projecting from the distal end of the preceding subsegment. The number of sensilla in a cluster varies with the subsegment, the distal subsegments usually having the larger clusters; details of size, number and arrangement differ among various taxa.

In *Speyeria cybele* a pair of ventral spines arises at the distal margin of each of the first four foretarsal subsegments, one spine on each side of the ventral midline (Fig. 4). Beneath each spine there is a cluster of sensilla on the next distal subsegment; no such cluster is present on the first subsegment. The two clusters on the second subsegment each consists of four sensilla; on the third and fourth subsegments each cluster has seven sensilla; on the fifth subsegment each consists of twelve sensilla. The setae (Figs. 5, 6) are 72 to 98.4 micra long and 7.2 to 9.0 micra

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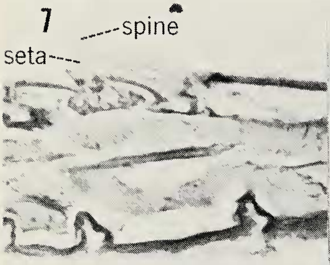

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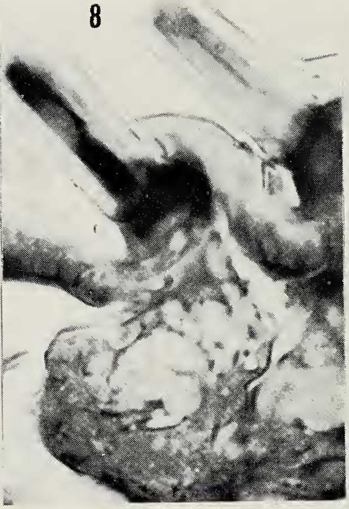

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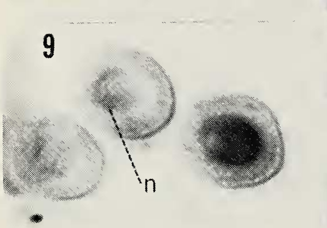

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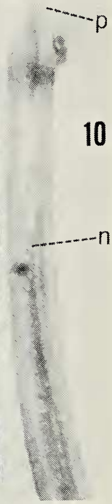

Fig. 7. Longitudinal section of foretarsus of S. *cybele* female, cut at 7 micra, stained with Mallory's triple stain and photographed at 100x, showing spine of first subsegment and two sensilla of second segment; only basal part of setae included.

Fig. 8. Same two setae and associated cells photographed at 1000x.

Fig. 9. Three setae in cross-section photographed at 1000x, showing position of dendrite (*n*) against wall of lumen.

Fig. 10. Longitudinal section through tip of seta photographed at 1000x, showing terminal papilla (*p*) and part of neural denrite (*n*).

in diameter at the base. In each clump the setae appear to grade in length, the longest being distal. The discs or mounds in which the setae are 12.0 to 14.4 micra in diameter and are placed from 4.7 to 9.5 micra apart.

Examination of serial sections cut longitudinally (Figs. 7, 8) shows that each disc is a hemispherical mound set in a depression of the cuticular surface and that the whole structure is a trichoid sensillum much as presented by Eltringham (1933), Snodgrass (1935: 515-518) and Dethier (1963: pl. 11), with trichogen cells and, in this case, with apparently three neuron cell bodies. The setae are nearly round in cross-section, hollow, the walls about 1.75 micra thick (Fig. 9). The dendrites of the neurons (n) extend along the dorsal wall of the setal lumen to its tip, where there is a tiny terminal papilla (*p*) (Fig. 10).

As noted previously, Minnich (1921) and Frings and Frings (1949, 1956) showed that pierids studied differ from nymphalids studied in that sensitivity to sucrose solutions is present on the foretarsi of the former but not of the latter butterflies. In addition, therefore, to the clumped sensilla on the female foretarsus, pierids would be expected to have trichoid sensilla individually scattered along the foretarsi of both sexes. Whole mounts of the foretarsi of *Pieris rapae* confirm this situation.

Scattered along the entire ventral surfaces of the foretarsi of both sexes are trichoid sensilla separated from each other by at least 24 micra. Similarly arranged sensilla are found also on the midtarsi (Fig. 11) but not on the hindtarsi of both sexes.

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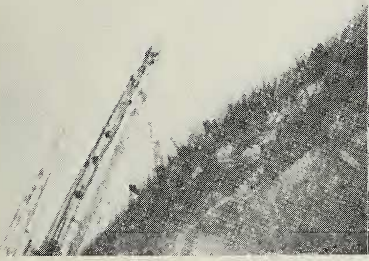

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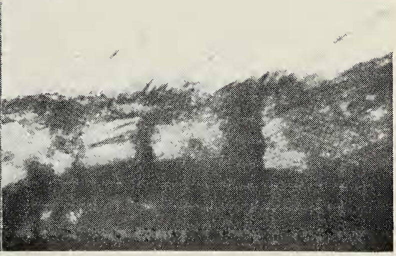

Fig. 11. Fifth midtarsal subsegment of *P. rapae* female photographed at 200x, showing ventral spines and scattered sensilla.

Fig. 12. Fifth foretarsal subsegment of *P. rapae* female photographed at 200x, showing clumped sensilla and paired spines.

Clumps of sensilla are present only on the foretarsi of females (Fig. 12). The discs are separated by 4.8 to 9.5 micra, otherwise these sensilla are structurally similar to the scattered sensilla. The setae are 55.2 to 60.0 micra long and 4 to 6 micra in diameter at the base.The two clumps on the fifth subsegment each consist of 12 sensilla, those on the fourth of four each and those on the third of two each; there are no clumps on the first or second subsegments.

The individually scattered sensilla are, as reported by Eltringham (1933) and others, the contact chemoreceptors apparently concerned with appreciations of solutions: confirmation has been provided by electrophysiological methods and there is correlation of structure and function. By the same token, the clumps of sensilla cannot be involved in detection of solutions, since these structures are present on the foretarsi of females shown not to possess on the foretarsi any sensitivity to fluids.

A clue to an additional foretarsal function in female butterflies was found in the work of Ilse (1937), who experimented with insect vision and color discrimination. She reported that ovipositing *Pieris brassicae* females, when placed on a green surface, reacted by rapidly drumming on the surface with the forelegs, but that no such reaction was elicited when the same females were placed on yellow or red surfaces. Ilse was content to interpret the drumming reaction as a sign that the butterfly recognized the color green, without wondering why recognition should be followed by such astonishing foreleg activity.

It occurred to me that the drumming reaction on the part of an ovipositing female might be an attempt by the butterfly to carry the visual recognition of green one step farther — the contact chemoreceptive identification of the correct larval foodplant. As a crude preliminary investigation of this line of thought, ovipositing females of *Pieris rapae, Colias philodice, Phyciodes tharos, Speyeria cybele* and *Euphydryas phaeton* were netted in the field. Wings were clamped together with paper clips and the butterflies were placed on various colored surfaces. When black, white or orange surfaces were used, no reaction was observed. When a green surface was used, a form of the drumming reaction was observed in every case. The two species of pierids performed drumming exactly as Ilse described it for *P. brassicae,* a repeated, rapid alternating movement of the forelegs against the surface. In the three nymphalids the reaction consisted of a single darting motion, first with one, then with the other foreleg.

In all cases the terminal tarsal subsegments were brought into contact with and scraped along the surface at the end of the stroke.

Each female was then placed upon various green leaves, including the leaf of her foodplant. Each new offering was always drummed. Three females belonging to two species followed what apparently was the butterfly's recognition of her foodplant by oviposition: two different females of *P. tharos* laid eggs on leaves of aster but did not do so on other leaves offered; one female of *C. philodice* oviposited on a clover leaf but not on any other offering.

My theory – certainly not proved at this time – is that the female butterfly, by drumming, abrades with the tarsal spines the surface of the leaf, releasing essential oils and enabling her to detect via the clumped trichoid sensilla the material characteristic of the plant. It seems probable that, during my rough observations, the females who did *not* follow drumming on the larval foodplant by ovipositing were not ready to do so for other reasons or were inhibted by having the wings restrained. In any event, it does not necessarily follow that just because a female comes into contact with and recognizes her foodplant that she will immediately and invariably oviposit. On the other hand, the instances of ovipositing observed may have been mere coincidence and may have had nothing to do with drumming.

If, however, the above explanation of drumming turns out to be correct, the clumps of trichoid sensilla on the female foretarsi are also explained and the function which can be performed by miniaturized foreleg only if it is complete and unfused has been found.

Experiments are currently under way in our laboratory and are expected to shed additional light on these questions.

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